A simple freezing device.

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The apparatus consists of a freezing chamber upon which the object is frozen, and two pails filled with ice and salt. The pails are so arranged that when either of them is raised the other is lowered, and in consequence, the cold brine, which forms as the ice melts, can be made to flow back and forth through the freezing chamber which is between the pails and connected with them by rubber tubing.

The freezing chamber is a hollow cylinder made of brass or of iron, nickel-plated both inside and outside. Fig. 1 shows a section of the chamber, actual size. The brine enters one of the tubes at the side and escapes by means of the other. Below the bottom of the chamber, *D*, the cylinder is furnished with a thread inside, by means of which a wooden plug, *C*, is screwed firmly against the bottom of the chamber. The wooden plug is held securely in the jaws of the microtome and prevents the conduction of too much heat from the latter to the freezing chamber.

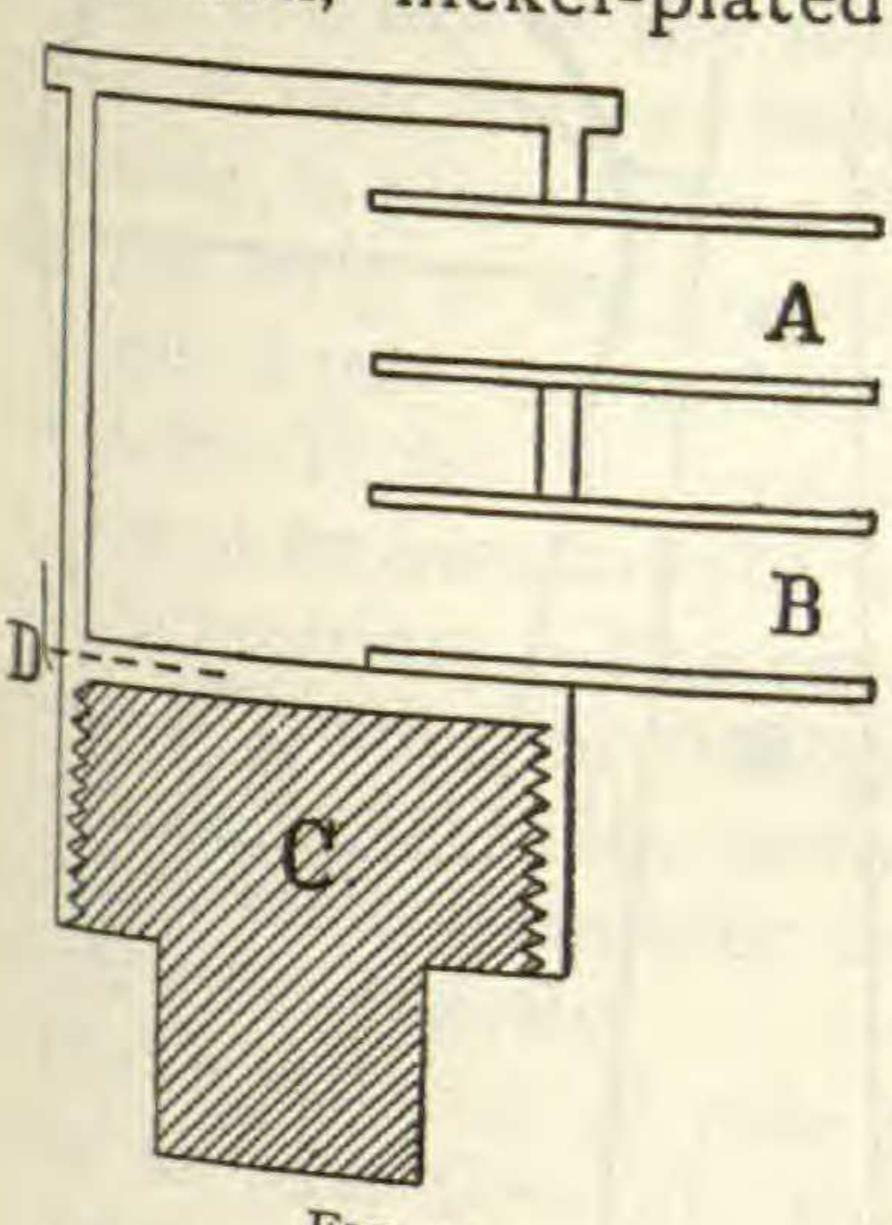

FIG. 1.

Large pails holding about five gallons are most convenient, since they contain ice enough to run the apparatus all day without being refilled. Smaller pails are just as serviceable if the apparatus is to be used for a short time only. Paper pails are best for the purpose, but ordinary wooden ones can be used. Each pail should be provided with a faucet capable of delivering a stream of water one-quarter of an inch in diameter; the faucet should be inserted about an inch from the bottom of the pail. The paper ice-water pails in common use answer the purpose perfectly.

In order to prevent the dirt, straw, etc., which usually adheres to the ice, from passing into the freezing chamber a piece of coarse wire netting should be bent into the form of

a hollow cylinder six inches long and four in diameter. The cylinder should then be stuffed with excelsior, and one end of a piece of glass tubing pushed into the center of the mass; the free end of the tube should then be connected with the faucet on the inside of the pail by a short rubber tube. The brine is then forced to filter through the tightly packed excelsior before entering the tube.

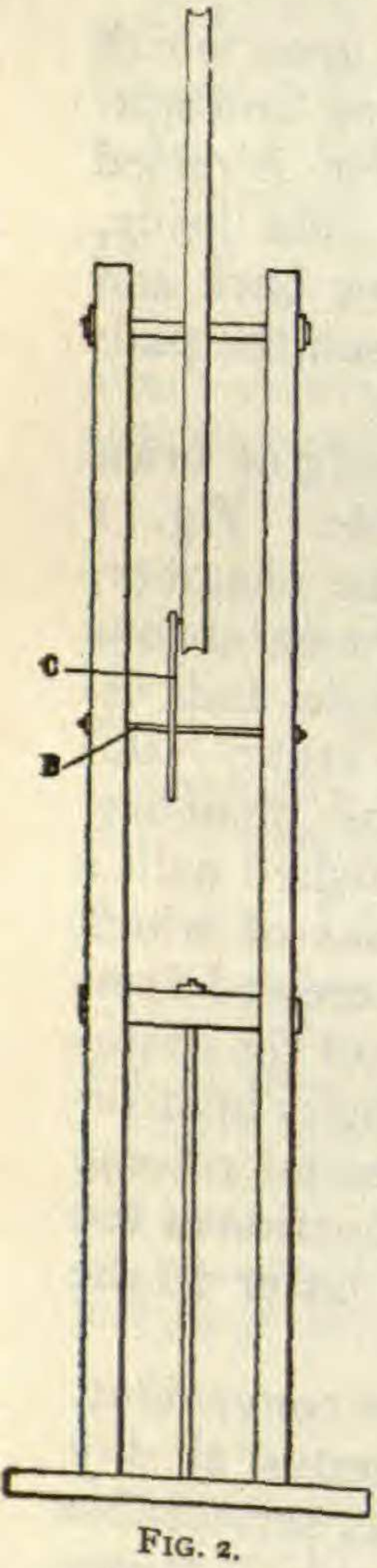

FIG. 2.

FIG. 3.

The standard upon which the pails hang is constructed of wood. It can best be understood by a reference to figures 2 and 3. Both figures are one-twelfth actual size. Figure 3 shows a side view of the standard. The diameter of the wooden wheel must be great enough to prevent the pails from striking the upright piece as they rise and fall. The rope which passes over the wheel is secured at its middle point by a nail driven through it into the wheel; the pails are fastened

to the rope by ordinary metal "snaps," so that they can be instantly detached or put on. The rope should be of such length that when the bottom of one pail is about level with the top of the other the bottom of the latter hangs an inch and one-half above the base of the standard.

The construction of the upright piece is evident from figure 2, which shows an end view of the apparatus. The two upright pieces are held together by a wooden cross-piece, from which a stout metal rod runs to the base; the axle of the wheel also aids in holding the two pieces together.

An automatic catch serves to retain the pails in the position shown in figure 3. Figures 4 and 5 show this contrivance in flat view, one-half actual size. It is made of sheet iron, one-quarter of an inch in thickness. The small hole at the top is for the screw which fastens it to the wheel. It has a single continuous slot, *D*, in which are two notches, *F* and *F;* the outlines of the slot and notches are dotted in the figures. A thin strip of copper, one inch wider than the sheet-iron strip, is placed upon the latter, and its edges bent around the iron strip so as to partially surround it. It is thus able to slide freely upon the iron strip, but it should not fit loosely enough to slide of its own weight when in an upright position. The copper strip, which is shaded in the figures, has a slot, *E*, which in its narrowest part has the same diameter as *D*, over which it fits exactly.

The metal rod *B* (see fig. 2) is made to pass through the middle of both uprights and through the slots *D* and *E*, in which it must be small enough to slide freely. The strip *C* is then secured to the wheel by a screw passing through the hole at the top. The screw should be small enough to permit the strip to swing freely. The strip is fastened to the wheel in such a way that when the wheel is turned so as to bring both pails on a level, the strip is vertical. It should be fixed

FIG. 4.

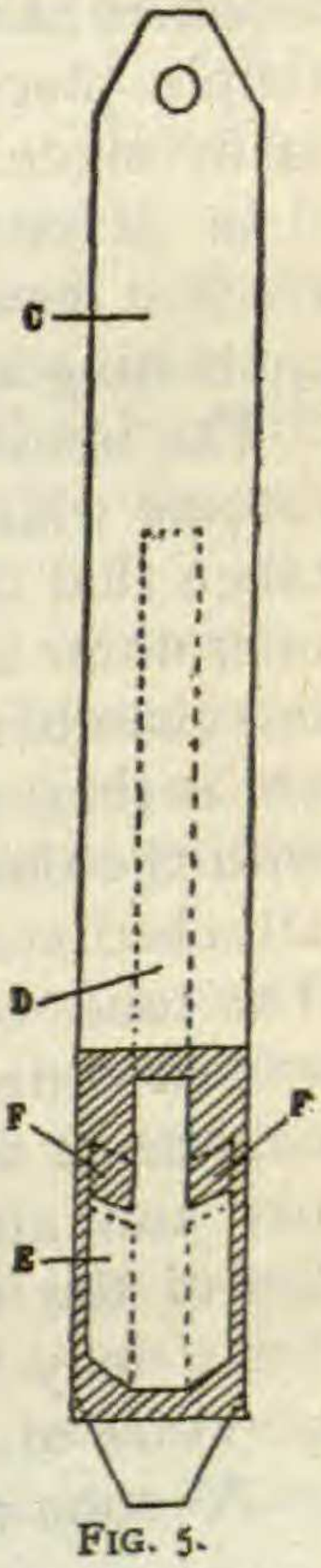

FIG. 5.

at such a height that when the pails are in the position shown in fig. 1, the metal rod, *B*, slips into the notch *F*, (fig. 4).

The weight of the upper pail (which is the heavier when the apparatus is in use) retains the rod in this position. The copper strip now has the position shown in figure 4. As the brine flows from the upper pail into the lower, the latter (which hangs one and one-half inches from the base of the standard), becomes the heavier and settles slowly down till it rests on the base. This causes the wheel to turn and the rod *B* (fig. 2) travels to the bottom of the slot *D* (fig. 5) carrying with it the copper strip, which takes up the position shown in fig. 5. When all the brine has passed into the lower pail the latter should be raised. As the pail raises the wheel turns and the rod *B* travels upward in the slot *D*, but passes over the notch *F*, which is now covered by the copper strip. It continues to travel upward, carrying the copper strip with it until the iron strip *C* reaches a vertical position. As the wheel continues to turn the rod *B* begins to descend in the slot *D* until it again drops into the notch *F*. As the heavier pail is now uppermost it must remain so until the lower pail becomes heavier, when the process is repeated. This very simple piece of apparatus answers its purpose perfectly, is easily made and cannot get out of order.

In order to use the apparatus both pails are filled with cracked ice and coarse salt in alternating layers, the cylinder containing excelsior resting on the bottom of the pail.

The freezing chamber is clamped into the jaws of the microtome which touch only the wooden plug. Care should be taken that the metal part of the chamber does not touch the microtome as otherwise heat will be conducted to the freezing chamber and freezing will be more difficult. The standard is then placed on the table at the left of the microtome, with the end facing the front of the table. The pails are then attached and made to assume the position shown in figure 3. The tubes of the freezing chamber should project at the left and be connected, one with each faucet by rubber tubing. A cold brine soon forms which flows through the freezing chamber and almost instantly freezes the material placed on the top of the chamber. By means of the faucets the flow of brine may be regulated, and this in turn regulates the temperature of the chamber.

As soon as the brine has all run into the lower pail the po-

sition of the two pails should be reversed. The person using the microtome does not need to rise from his chair to accomplish this, since he can easily reach the pail nearest him and raise or lower it as necessity requires. The weight to be lifted is simply the weight of the brine, since the weights of the two pails with their content of ice and salt balance each other. The shifting of the pails therefore occupies only the left hand, leaving the right free. The falling of the lower pail indicates that most of the brine has run through. If a more accurate indication is needed one may insert in one of the rubber tubes a glass T-tube with a cork at the top of the free arm of the tube. A black feather may be fixed to the cork in such a way that the end dips in the current and shows by its deflection the direction and force of the flow. When too much brine has accumulated the temperature rises and freezing proceeds too slowly; a part must then be siphoned off. Usually this happens once, or at most twice, during the course of one day's use of the apparatus. The addition of more ice is very seldom necessary.

If the operation of freezing is interrupted for less than half or three-quarters of an hour the faucets should be nearly closed. Objects already frozen will remain so under these conditions as long as the brine continues to flow. If the apparatus is to be left for a longer time the brine should all be drawn off and thrown away, and the faucets closed. A moment's flow will render the apparatus ready for use again.

During a year's test in cutting both plant and animal tissues this apparatus has proven entirely satisfactory. Its principal advantages are as follows:

1. It is simple, inexpensive and cannot get out of order.

2. The material cannot alternately thaw and freeze (as is the case when it is frozen by the ether or carbonic-acid-gas method), but remains at a constant temperature and of uniform consistency.

3. The danger of over-freezing, which has proved a serious defect in the carbonic-acid-gas method, is entirely obviated. On the other hand, no difficulty is experienced in freezing, as is often the case with the ether method.

4. It can be applied to any microtome which has a horizontal movement of knife or object carrier. In most cases no previous embedding is necessary but fresh material, or that which is preserved in aqueous media, may be placed in a syrupy

solution of gum arabic[1] and frozen at once. Tissues sectioned in this way are practically unaltered. Delicate tissues and those containing large air cavities can be sectioned with the greatest ease. The damage wrought in many tissues by dehydration are entirely obviated by the freezing method.

Frozen sections may be mounted in glycerin jelly or any aqueous medium. If mounted in glycerin, it will be found convenient to ring the cover with a thick solution of gum arabic. A glycerin-gum is then formed around the edge of the cover and in a short time is dry and hard on the outside. A ring of Canada balsam or cement may then be applied and the mount is as permanent as it is possible to make a glycerin mount. Frozen sections may be fixed to the slide for staining purposes by Fol's method (see Lee; Microtomist's Vade-Mecum, 218, 1893).

When delicate parts are present, such as spores, etc., which are apt to fall out of the section, the following method can be used with entire success.

A gelatin solution is made as follows: The best gold label gelatin is selected and carefully brushed clean and made up according to the following formula: gelatin, 32 parts; distilled water, 48 parts; carbolic acid, 1 part. Dissolve the gelatin in a closed vessel over a water bath; add the carbolic acid and mix until it is thoroughly dissolved, and then add the white of one egg to clarify.[2]

The object to be cut is placed for a short time in the melted gelatin and quickly washed in warm water (about 90° Fahr.) to remove the gelatin from the outside. It is then placed at once in water at room temperature. This sets the gelatin with which the object is now infiltrated, and the latter may be sectioned at once in gum arabic or preserved in two per cent. formalin until needed. If sections cut in this way are placed on a slide and a little glycerin added over a water bath, a glycerin jelly is formed and the addition of a cover and a ring of cement completes the process. If the sections are taken from the knife in the gum in which they are cut and placed at once on the slide and subjected to the process just mentioned, a glycerin-jelly-gum is formed which makes an excellent mounting medium.

A simpler form of the above apparatus is shown in figure

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[1] As some of the preservatives ordinarily added to gum arabic attack the knife, formalin is recommended for this purpose.

[2] For this formula I am indebted to Mr. N. N. Mason of Providence, R. I.

6.[3] The larger pail is filled with ice and salt and the smaller one catches the brine which is poured back into the larger pail. A stop-cock at the lower pail regulates the flow; the former is fastened to the pail by a wooden clothes pin.

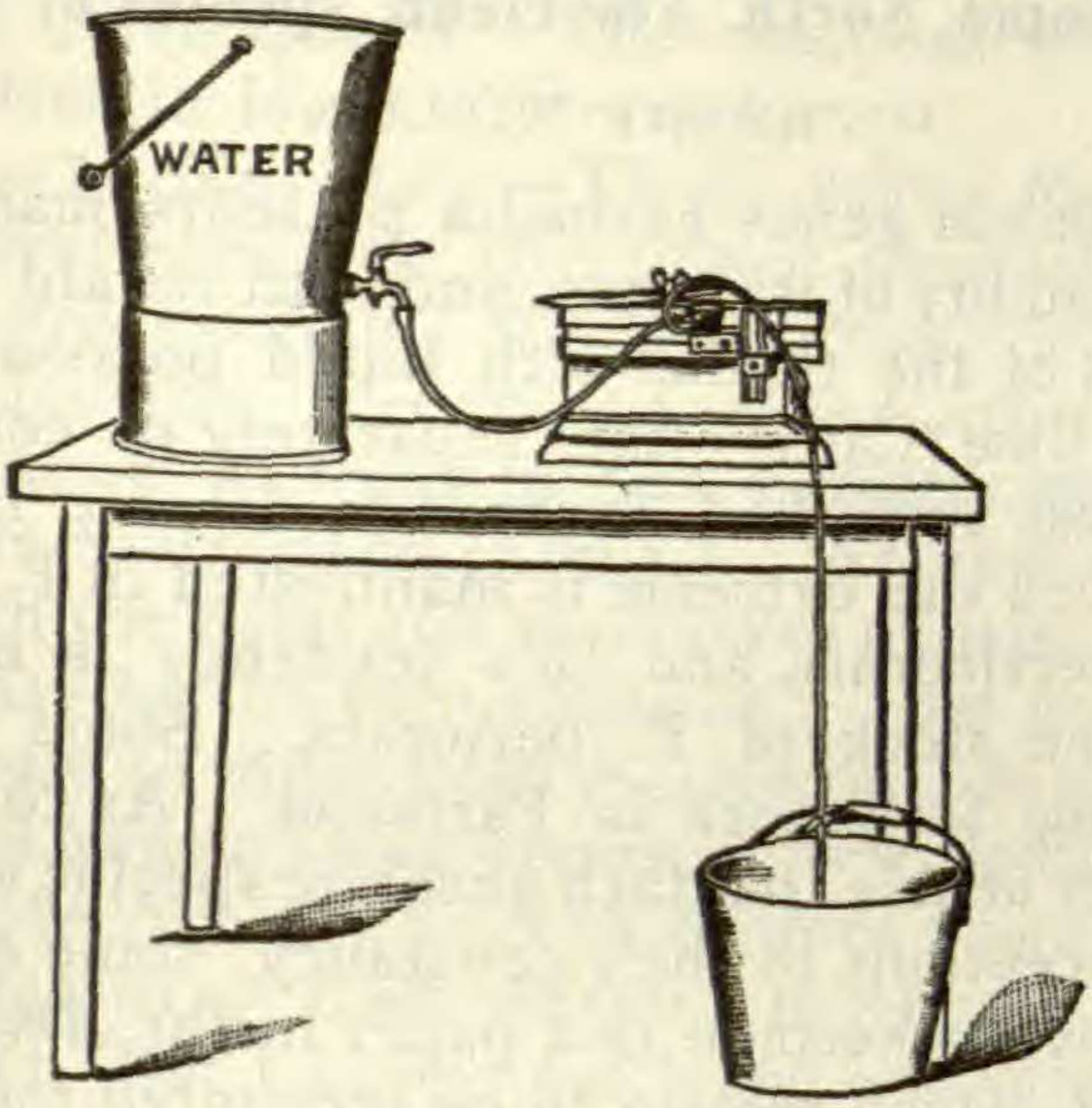

FIG. 6.

An inexpensive substitute for this form of the apparatus may be quickly made. A large wooden pail may be bored near the bottom, a cork inserted and pierced by a glass tube. A piece of brass pipe, one inch in diameter, with a thread at the end may be sawed off an inch and a half from the end and converted into a freezing chamber by soldering in the brass discs and inserting two straight tubes at the side. A glass tube drawn to the required aperture may be used in the place of the stop-cock at the lower pail and other tubes of different apertures may be quickly substituted.

Providence, R. I.

[3]For the use of this cut I am indebted to the courtesy of the Bausch & Lomb Optical Co., Rochester, N. Y.